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Direct observation of vibrational coherence in bacterial reaction centers using femtosecond absorption spectroscopy (photosynthesis/charge separation/mutant)
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ABSTRACT It is shown that vibrational coherence modulates the femtosecond kinetics of stimulated emission and absorption of reaction centers of purple bacteria. In the DLL mutant of Rhodobacter capsulatus, which lacks the bacteriopheophytin electron acceptor, oscillations with periods of 500 fs and possibly also of 2 ps were observed, which are

associated with formation of the excited state. The kinetics, which reflect primary processes in Rhodobacter sphaeroides R-26, were modulated by oscillations with a period of =700 fs at 796 nm and -2 ps at 930 nm. In the latter case, at 930 nm, where the stimulated emission of the excited state, P, is probed, oscillations could only be resolved when a sufficiently narrow (10 nm) and concomitantly long pump pulse was used. This may indicate that the potential energy surface of the excited state is anharmonic or that low-frequency oscillations are masked when higher frequency modes are also coherently excited, or both. The possibility is discussed that the primary charge separation may be a coherent and adiabatic process coupled to low-frequency vibrational modes.

The quantum efficiency of the initial charge-separation process in the reaction center of photosynthetic bacteria is near unity because of the combination of the extreme rapidity of forward electron transfer and the low probability of back reaction. In conventional electron-transfer theory (1, 2), it is assumed that vibrational relaxation takes place on a time scale faster than electron transfer and that electron transfer is essentially nonadiabatic. It may be questioned whether these assumptions are justified for the ultrafast initial reactions taking place in the bacterial reaction center. In particular, electron transfer from an excited state that is not completely vibrationally relaxed (3) may be at the origin of the high quantum yield of charge separation. In this case, vibrational coherence of modes coupled to electron transfer is not necessarily lost on the time scale of the reaction. Recently, theoretical studies have appeared in which it has indeed been suggested that vibrational coherence or coherence in the electronic coupling, or both, play a role in primary electron transfer (4-7).

If the vibrational relaxation takes place on a time scale that is not significantly faster than electron transfer, low-frequency (<100 cm-1) vibrations may interfere with the

charge-separation reaction. When such vibrations are coherent, oscillations may in principle be observed in the optical transients of the involved electronic state(s). Here we report the observation of coherent processes associated with the excited state in the photosynthetic reaction center.

The reaction center of purple bacteria, which are used in this study, normally contains four bacteriochlorophylls [two

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of which display strong dimeric interaction and are designated P (for "special pair") and two of which, B, and BM, are more monomer-like], two bacteriopheophytins (H, and HM). and two quinones (QA and QB) bound to the protein subunits L and M (8, 9). The lower-exciton excited state of the dimer, P*, is the precursor of electron transport; the charge-separated state P*Hi is formed in 0.7-3.5 ps, depending on the species and temperature (10-16). Until now, femtosecond studies have not revealed any deviations from smoothly decaying functions in the kinetics of P*. Recently, however, complicated absorption kinetics were reported in the region of the B absorption band at room temperature (15, 16). These data were analyzed in terms of a linear reaction scheme where the state P+B is the short-lived intermediate during the transfer of the electron to H.

We have collected femtosecond transient spectra and kinetics in the near infrared with a high signal-to-noise ratio and high spectral and temporal resolution at cryogenic and at room temperatures. In this paper we will focus on the oscillatory features of the kinetics. We studied reaction centers of Rhodobacter sphaeroides R-26 and the DLL mutant of Rhodobacter capsulatus. The latter mutant lacks the bacteriopheophytin acceptor H_1 (17) and serves as a model

for studying the kinetic evolution of the dimer excited state, P*, as the population of this state essentially does not decay on the time scale of a few picoseconds (18). The reaction centers of R. sphaeroides and R. capsulatus wild type display virtually similar spectral (19) and kinetic (ref. 14; J.-L.M. and J. Breton, unpublished results) characteristics, and hence the two types of reaction centers may well be compared.

MATERIALS AND METHODS

Reaction centers of the carotenoid free strain R-26 of R. sphaeroides were prepared as described (10). The quinone acceptor QA was reduced by addition of 50 mM dithiothreitol (Em-330 mV) to ensure complete back reaction between flashes. Preparation of chromatophores of R. capsulatus mutant DLL that were devoid of antenna pigments is described elsewhere (20).

For cryogenic experiments, samples were diluted in glycerol (60% vol/vol) and were cooled in the dark to 100 K or to 10 K in a convection cryostat. For room temperature measurements, the sample was continuously moved perpendicular to the optical beams. The optical density of the samples was about 0.5 at 870 nm (1-mm optical path length). Generation of the 50-fs pulses at 620 nm was essentially as described (11). The signal-to-noise ratio and the temporal resolution of the overall system were considerably enhanced by modifications in the amplification and compression chain (unpublished data). Pump pulses were centered at 870 nm and had a pulse width of either 45 fs (25-nm spectral width) or, when explicitly mentioned, 80 fs (10-nm spectral width). The